

WHAT IS CLAIMED IS

1. A method of producing phage particles, the method comprising:
providing a set of host cells, wherein each of the host cells of the set comprises
 - a) a first expression unit comprising
 - (1) a first open reading frame, encoding a first polypeptide comprising
 - (i) an amino acid sequence to be displayed on a phage and
 - (ii) a portion of a phage coat protein of a filamentous phage, wherein the portion of the phage coat protein physically associates with phage particles, and
 - (2) a first promoter operably linked to the first open reading frame, and
 - b) a second expression unit comprising:
 - (1') a second open reading frame, encoding a second polypeptide comprising a portion of the phage coat protein, and
 - (2') a second promoter operably linked to the second open reading frame, wherein the second promoter is regulatable; and

maintaining the set of host cells under a first condition, wherein phage particles that include amino acid sequences to be displayed are produced.

- 20 2. The method of claim 1, wherein the amino acid sequence to be displayed varies among cells of the first set.

- 3. The method of claim 2, wherein the second polypeptide is invariant for all host cells of the set.

- 25 4. The method of claim 1, wherein the second polypeptide does not include a non-phage sequence of greater than five amino acids in length.

- 5. The method of claim 1, wherein the first condition increases activity of the
30 regulatable promoter relative to a reference condition, and the phage particles produced by

the first set of host cells are characterized by a first average number of copies of the first polypeptide.

6. The method of claim 1, wherein the first condition decreases activity of the
5 regulatable promoter relative to a reference condition, and the phage particles produced by the first set of host cells are characterized by a first average number of copies of the first polypeptide.

10 7. The method of claim 1, wherein the first expression unit is a component of a nucleic acid element that further comprises a phage origin of replication and a phage packaging signal.

8. The method of claim 1, wherein the first polypeptide comprises an immunoglobulin variable domain sequence.

15 9. The method of claim 8, wherein the first expression unit further comprises an additional open reading frame that encodes a polypeptide comprising an immunoglobulin variable domain sequence, compatible with the immunoglobulin variable domain sequence in the first polypeptide.

20 10. The method of claim 1, wherein the second polypeptide comprises a mature full-length coat protein.

25 11. The method of claim 1, wherein the portion of the coat protein in the first and second open reading frame is a portion of a gene III protein.

12. The method of claim 11, wherein the gene III protein is a wild-type gene III protein.

30 13. The method of claim 11, wherein the gene III protein is a mutant of gene III protein that physically associates with phage particles less efficiently than wild-type.

14. The method of claim 1, wherein the portion of the coat protein in the first or second open reading frame is encoded by at least one synthetic codon.

5 15. The method of claim 1, wherein activity of the second promoter is regulated by an agent, and the first condition includes presence of the agent.

16. The method of claim 15, wherein the second promoter regulatable by the lacI repressor.

10 17. The method of claim 1, wherein the first promoter is a phage promoter.

18. The method of claim 17, wherein the phage promoter is a promoter naturally associated with an open reading frame encoding phage coat protein.

15 19. The method of claim 1, further comprising:
selecting a subset of the phage particles produced by the host cells,
introducing nucleic acid from phage particles of the subset into a second set of bacterial host cells,
maintaining at least two host cells of the second set under a second condition that
20 results in a different level of activity of the regulatable, second promoter than the first condition, wherein phage particles produced by the second set of host cells are characterized by a second average number of copies of the first polypeptide physically attached to the phage, wherein the second average number of copies is different from the first average number of copies.

25 20. The method of claim 19, wherein the second average number of copies is less than the first average number of copies.

30 21. The method of claim 19, wherein the selecting comprises contacting phage to a target, and separating phage that bind the target from phage that do not bind the target.

22. The method of claim 19, further comprising selecting a subset of the phage particles produced by host cells of the second set.

23. A host cell comprising:

5 a) a first expression unit comprising (1) a first open reading frame and (2) a first promoter operably linked to the first open reading frame, wherein the first open reading frame encodes a first polypeptide comprising (i) an amino acid sequence to be displayed on a phage and (ii) a portion of a phage coat protein, the portion of the phage coat protein being capable of physically associating with phage particles, and

10 b) a second expression unit comprising (1') a second open reading frame and (2') a second promoter that is regulatable and operably linked to the second open reading frame, wherein the second open reading frame encodes a second polypeptide comprising a portion of the phage coat protein, the portion of the phage coat protein being capable of physically associating with phage particles.

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24. The host cell of claim 23, wherein the first expression unit is a component of a nucleic acid element that further comprises a phage origin of replication and a phage packaging signal.

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25. The host cell of claim 23, wherein the first expression unit and the second expression unit are on separate nucleic acid molecules.

26. A nucleic acid comprising:

a) a first expression unit comprising (1) an open reading frame and (2) a first promoter operably linked to the open reading frame, wherein the open reading frame encodes a first polypeptide comprising (i) an amino acid sequence to be displayed and (ii) a portion of a phage coat protein, the portion of the phage coat protein being capable of physically associating with phage particles, and

b) a second expression unit comprising a (1') second open reading frame and (2') a second promoter that is regulatable and operably linked to the second open reading frame, wherein the second open reading frame encodes a second polypeptide comprising a portion of the phage coat protein, the portion of the phage coat protein being capable of physically associating with phage particles.

27. The nucleic acid of claim 26, wherein the first promoter is a phage promoter and the second promoter is a lac promoter.

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28. A phage genome that comprises the nucleic acid of claim 26.

29. A plurality of phage particles produced by the method of claim 1.

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30. A library of host cells, the library comprising a plurality of host cells, each cell being according to claim 23, wherein the amino acid sequence to be displayed varies among cells of the plurality, and the host cells of the plurality collectively encode between 10^3 to 10^{11} different amino acid sequences to be displayed.

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31. A library of phage particles, the library comprising a plurality of phage particles that comprise a phage genome of claim 28, wherein the amino acid sequence to be displayed varies among phage particles of the plurality, and the phage particles of the plurality collectively encode between 10^3 to 10^{11} different amino acid sequences to be displayed.

32. A phagemid comprising:

- a) an open reading frame that encodes a polypeptide comprising an amino acid sequence to be displayed and a portion of a phage coat protein, wherein the amino acid sequence to be displayed is a heterologous sequence,
- 5 b) a promoter, operably linked to the open reading frame, wherein the promoter is (i) a phage promoter or (ii) a promoter that has less than 50% of the activity of the *lac* promoter in Luria Broth at 37°C,
- c) a phage origin of replication, and
- d) a phage packaging signal.

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33. A kit comprising:

- (a) the phagemid of claim 32 or a phage particle or cell that contains the phagemid; and
- 15 (b) an isolated nucleic acid that comprises a nucleic acid sequence that includes an open reading frame that encodes a polypeptide comprising a portion of a phage coat protein and a regulatable promoter, operably linked to the open reading frame, or a phage particle or cell containing the nucleic acid.

34. A phagemid comprising:

- 20 a display cassette configured to receive a sequence encoding an amino acid sequence to be displayed; and
 - a sequence encoding at least a portion of a phage coat protein; and
 - a promoter that is identical, or substantially identical to an endogenous phage promoter, or includes a sequence that hybridizes to a strand of an endogenous phage promoter, the promoter being operably linked to the display cassette such that a transcript can be produced that includes a sequence inserted into the display cassette and the sequence encoding at least a portion of the phage coat protein.

35. A phagemid comprising:

a coding sequence encoding a polypeptide that comprises a first amino acid sequence to be displayed and at least a portion of a phage coat protein; and

5 a promoter that is identical, or substantially identical to an endogenous phage promoter, or includes a sequence that hybridizes to a strand of an endogenous phage promoter, the promoter being operably linked to the coding sequence.

36. The phagemid of claim 35, further comprising a second coding sequence that encodes a second amino acid sequence to be displayed, wherein the second amino acid sequence is 10 not attached to a portion of phage coat protein, but can associate with the first amino acid sequence.

37. A method of providing phage particles that display a heterologous amino acid sequence, the method comprising:

15 providing a host cell that includes the phagemid of claim 32, and a genome of a helper phage, the genome comprising a regulatable promoter operably linked to a sequence encoding a coat protein whose abundance in the cell modulates incorporation of the amino acid sequence to be displayed into phage particles; and

20 maintaining the host cell under conditions, whereby phage particles that package the phagemid are produced.

38. The method of claim 37 wherein the conditions are selected to alter activity of the regulatable promoter relative to a reference activity level of the regulatable promoter.